were allocated into four groups (n=9 per group): a) control (explants in DMEM), b) MPA treatment (control for drug), c) inflammation (treatment with interleukin-1β (IL-1β)), and d) inflammation followed by inhibition with MPA (IL-1β+MPA). The MPA (10-7uM) was added following 24 hours of equilibration time in the MPA group. The pro-inflammatory cytokine IL-1β (10ng/ml) was added to the culture media to initiate inflammation in the IL-1β group. For the IL-1β+MPA group, IL-1β was added following the equilibration time and MPA (10-7uM) was added 24 hours later. All explants were harvested at 48 hours and snap frozen in liquid nitrogen and later used for mRNA analysis using real time qPCR. Matrix metalloproteinase (MMP)-1, MMP-3, and MMP13 were analyzed in the explants. In addition, one set of fat pad and synovial explants from each limb was tested for cellular viability using SYTO13 green fluorescent nucleic acid stain (Invitrogen). ANOVA with Holm-Sidak's post-hoc analysis was used to determine differences in mRNA expression between groups, using Prism 6 Graph Pad software.

Results: Prior to being exposed to the experimental conditions, the cells in explants from the synovium and fat pad were stained to ensure they were viable (Figure 1). The mRNA expression levels for MMP-3 and MMP-13 were suppressed in fat pad tissue when treated with MPA following induction of inflammation ($p<0.05$) (Figure 3). There were similar trends in synovium tissue for MMP-3 and MMP-13, however not significant potentially due to considerable animal variation (Figure 2). Interestingly, MMP-1 exhibited a trend in this pattern of mRNA suppression with MPA in both the fat pad and synovium tissue, although significance was not noted (Figure 2A and 3A).

Conclusions: Early intervention with 10-7uM MPA was successful in blocking inflammation and suppressing some degradative proteinases such as MMP-3 and MMP-13 after induction of inflammation in select tissues of the knee joint. Interestingly, MMP-1 mRNA expression did not show a significant repression with MPA. More detailed analysis revealed that high responder animals in the synovium tissue were not the same as the high responder animals in the fat pad, thus indicating that there was tissue specific variation. Different doses of MPA and longer treatment times are essential for future studies.

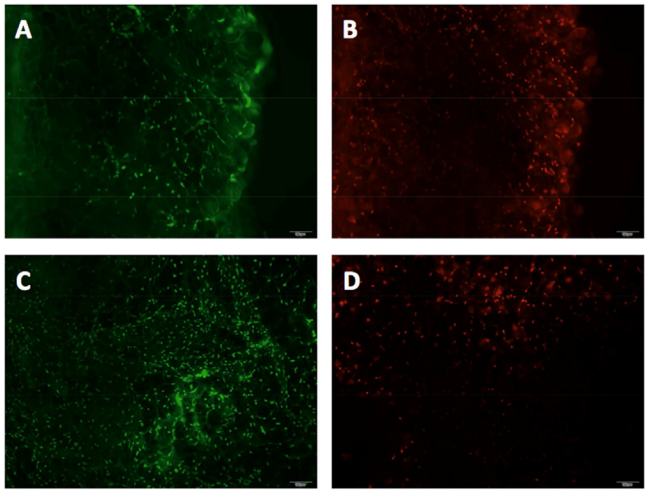

Figure 1. Live (green) and dead (red), respectively, cell staining in fat pad (A, B) and synovium (C, D) tissue.

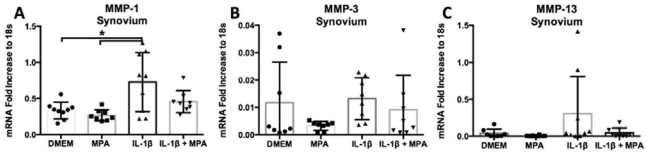

Figure 2. Analysis of mRNA expression of MMP-1 (A), MMP-3 (B), and MMP-13 (C) in synovium tissue explants.

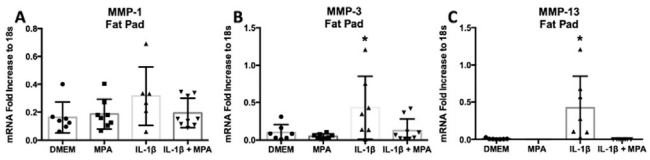

Figure 3. Analysis of mRNA expression of MMP-1 (A), MMP-3 (B), and MMP-13 (C) in fat pad tissue explants.

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MACROPHAGE PHENOTYPE MODULATION TO CONTROL SYNOVIAL INFLAMMATION

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Purpose: Macrophages are involved in the development of osteoarthritis (OA). Upon receiving cues from their microenvironment they can polarize towards a pro-inflammatory (M1) or anti-inflammatory (M2) phenotype, where the latter can be further divided into an M2a, M2b or M2c subtype. A potential strategy to control joint inflammation would be modulating the polarization state of macrophage phenotypes. The aim of this study was to evaluate compounds from various medication groups that are all described in literature to have inflammation modulatory effects, on human osteoarthritic synovial explants and on primary macrophages polarized to M1, M2a and M2c.

Methods: End-stage OA synovial explants were obtained from a patient who underwent total knee replacement (male, 66Y). To simulate acute inflammation, half of the explants were pre-stimulated for 24h with IFNγ+TNFα. Bone morphogenetic protein 7 (BMP-7), Rapamycin (Rapa), Dexamethasone (Dex) and Pravastatin (Prava) were added in three doses to the culture medium. The explants were cultured for an additional 3 days and assessed for IL6, CCL18 and IL1B gene expression. Primary human monocytes were isolated and pooled from 11 buffy coats (male donors, 46±16Y) by Ficoll density separation and CD14+ selection. The monocytes were cultured in monolayer in X-VIVO 15 medium and stimulated for 3 days with IFNγ+TNFα to obtain an 'M1'-like macrophage phenotype, stimulated with IL-4 to obtain 'M2a' and IL-10 to obtain an 'M2c'-like subtype. Protein production of IL-6 was considered as an M1 marker and CCL18 as an M2 marker, both measured with ELISA. The experiments were conducted three times with biological triplicates.

Results: In end-stage OA synovium, BMP-7 and Prava increased IL6 and CCL18 gene expression, whereas Dex reduced IL6. Rapa reduced CCL18, but only when the synovium was stimulated with inflammatory factors. All compounds reduced IL1B, but was increased in stimulated synovium except when treated with Dex. Since synovium contains various cell types, including all subtypes of macrophages, the compounds were cultured on isolated macrophages polarized to M1, M2a and M2c to further understand the effects of the compounds on macrophage subtypes. On M1 macrophages, BMP-7 had no effect regarding IL-6 production, Rapa and Prava reduced IL-6 and Dex strongly decreased IL-6 production. Rapa and Dex additionally reduced CCL18 production to some extent. On M2a macrophages, BMP-7 increased CCL18, Rapa reduced IL-6 and CCL18, whereas Dex increased both IL6 and CCL18 production. Prava had no effect on M2a macrophages. In M2c macrophages. BMP-7 increased CCL18 in a dose dependent manner, Rapa increased IL6 while decreasing CCL18 and Dex increased both IL6 and CCL18 production.

Conclusions: BMP-7, Rapa, Dex and Prava were all able to modulate the behavior of monocyte-derived macrophages cultured in monolayer. The modulatory capacity seemed to depend on macrophage phenotype. The effects of these compounds on synovial explants can be partially explained by the effects seen on macrophages. The effect of Dex on synovium was also seen in the monolayer cultures, where a suppression of M1 macrophages was seen without sacrificing M2a macrophages. BMP-7 tended to stimulate IL-6 and CCL18 which reflected the effects seen in the monolayer cultures. Prava had a subtle modulatory effect in both the explants and monolayers. Rapa decreased CCL18 in both stimulated synovium and in isolated macrophage cultures. These results indicate that compounds can modulate the behavior of synovial macrophages and thereby inflammation in a joint. To elaborate the actual modulatory effects of these compounds on synovial macrophages, in-depth analysis should be conducted to provide more insight on a potential treatment regimen to modulate macrophage phenotype.